influx via NCX. Thus, YM-244769 has therapeutic potential as an efficient renoprotective drug.

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Vascular Na^+/Ca^{2+} Exchanger Type-1 Contributes to Hypertension in Pseudohypoaldosteronism Type II and Cushing's Syndrome Models

Satomi Kita[1], Shinichi Uchida[2], Issei Komuro[3], Takahiro Iwamoto[1].
[1]Fukuoka Univ Faculty of Medicine, Fukuoka, Japan, [2]Tokyo Medical and Dental Univ, Tokyo, Japan, [3]Osaka Univ Graduate School of Medicine, Osaka, Japan.

Pseudohypoaldosteronism type II (PHAII) is an autosomal dominant disease characterized by hypertension due to increased renal salt reabsorption. Cushing's syndrome is associated with excessive cortisol secretion by ectopic ACTH producing tumor and may result in hypertension. Here we examined the role of Na^+/Ca^{2+} exchanger type-1 (NCX1) in hypertension of these model mice using specific NCX inhibitors and genetically engineered mice. NCX inhibitors lowered arterial hypertension in WNK4 mutant knockin mice (presenting the phenotype of PHAII) and chronically ACTH-administered mice. Furthermore, heterozygous knockout of NCX1 was resistant to development of hypertension in these model mice, whereas vascular overexpression of NCX1 accelerated their hypertension. Since NCX inhibitors reversed the cytosolic Ca^{2+} elevation and vasoconstriction induced by nanomolar ouabain, circulating endogenous cardiac glycosides may be involved in hypertension of these model mice. Thus, vascular NCX1 contributes to hypertension in PHAII and Cushing's syndrome, and NCX inhibitors might be therapeutically useful for their hypertension.

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Reverse Mode of the Sodium Calcium Exchanger is Enhanced in Malignant Hyperthermia Susceptible Skeletal Muscle

Jose M. Eltit[1], Nancy Linares[2], Isaac N. Pessah[3], P.D. Allen[1], **Jose R. Lopez**[1].
[1]Brigham and Women's Hospital, Boston, MA, USA, [2]CBB, IVIC, Caracas, Venezuela, Bolivarian Republic of, [3]University of California at Davis, Davis, CA, USA.

Intracellular Ca^{2+} concentration $[Ca^{2+}]_i$ and intracellular Na^+ concentration $[Na^+]_i$ were elevated in swine (RyR1-R615C) and rodent (RyR1-R163C) MH susceptible (MHS) compared to Wt (MHN) muscle fibers. In both MHN and MHS muscle fibers stepwise reduction of external $[Na^+]$ gradually increased $[Ca^{2+}]_i$, which could be prevented by removal of extracellular Ca^{2+} ($[Ca^{2+}]_{ext}$). Disruption of the T-system by glycerol treatment also reduced the magnitude of the $[Ca^{2+}]_i$ elevation induced by Na^+ free solution in both groups. Administration of KBR7943 reduced $[Ca^{2+}]_i$ in MHN and MHS muscle fibers, and ameliorated the magnitude of the elevation of $[Ca^{2+}]_i$ observed during a MH episode. However, YM-2444769 an NCX blocker that preferentially inhibits NCX3 reverse mode did not reduce $[Ca^{2+}]_i$ in either MHN or MHS muscle fibers at rest, but did reduce the amplitude of the elevation $[Ca^{2+}]_i$ induced by halothane in R163C MHS muscle fibers. These results support the existence of a functional NCX reverse mode in pig and mouse skeletal muscle, which appears to be enhanced in MHS muscle fibers, and shows that the majority of NCX is localized in the T-tubule. They further show that NCX in its reverse mode contributes to the elevation of $[Ca^{2+}]_i$ in MHS muscle during exposure to halothane.

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Atrial-Specific NCX KO Mice Reveal Dependence of Sinoatrial Node Pacemaker Activity on Sodium-Calcium Exchange

Sabine Groenke[1], Eric D. Larson[2], Haruko Nakano[3], Atsushi Nakano[3], Catherine Proenza[2], Kenneth D. Philipson[1], Joshua I. Goldhaber[4].
[1]David Geffen School of Medicine, Los Angeles, CA, USA, [2]University of Colorado, Denver, CO, USA, [3]University of California, Los Angeles, Los Angeles, CA, USA, [4]Cedars Sinai Heart Institute and David Geffen School of Medicine at UCLA, Los Angeles, CA, USA.

The cardiac sodium-calcium exchanger (NCX1) is hypothesized to play a major role in sinoatrial node (SAN) pacemaker activity. To test this hypothesis directly, we used cre/loxP technology to generate an atrial-specific knockout (KO) of NCX1 in mice using the sarcolipin promoter. At 12 weeks, there is no evidence of NCX1 in the atrium or SAN by immunostaining or immunoblot. KO mice exhibit atrial dilation and ventricular hypertrophy with mild ventricular dysfunction. On electrocardiography, KO mice have no P waves and a relatively slow junctional escape rhythm (231 $\pm$ 16 bpm, n = 4) compared to normal sinus rhythm in WT (422 $\pm$ 63 bpm, n = 2; p = 0.01). Furthermore, recordings of cardiac electrograms in Langendorff-perfused hearts show no evidence of atrial activity in KO. In patch clamped SAN cells isolated from KO mice, there is no NCX activity in response to caffeine-induced SR Ca^{2+} release. L-type Ca^{2+} current is decreased in KO by ~50% but there is no significant difference in funny current (I_f) amplitude between WT and KO. Spontaneous action potentials (APs) are absent in KO, even after application of isoproterenol (ISO, 1μM). However, we were still able to evoke APs in patch clamped KO cells under current clamp conditions, indicating that KO cells are capable of electrically stimulated depolarization. The maximum diastolic potential (MDP) was slightly more depolarized in KO (- 57 $\pm$ 2.0 mV) compared to WT (- 70 $\pm$ 2.5 mV, $p < 0.001$), which could theoretically reduce spontaneous activity. However, reducing extracellular K^+ to lower the MDP in KO to WT values failed to restore rhythmic pacemaker activity. Thus, we conclude that NCX1 is required for normal pacemaker activity in the murine SAN.

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A New view of Insulin Action

Richard D. Moore.
retired from SUNY, Platsburgh, NY, USA.

Insulin not only stimulates the Na-K-pump,but by stimulating the Na:H exchange pump increases intracellular pH, pHi. Fidelman et al (1982) found, as predicted by thermodynamic theory, the effect of insulin on glycolysis varied linearly with log[Na]o with the insulin effect being converted from stimulation of glycolysis to a 51% inhibition at 0.12 mM Nao.

Zierler & Rabinowitz (1964) demonstrated in the forearm of human males that levels of insulin too low to affect glucose uptake, as little as 38 micro units per ml, was sufficient to stimulate the Na-K-pump, as reflected by increased potassium uptake, while producing no effect upon glucose uptake. This one experiment forces us to realize that the main action of insulin is not to regulate blood glucose levels, but to regulate the Na-K-pump and Na:H exchange. Since the Na-K-pump uses about 25% of the ATP production in a resting muscle, stimulation of the Na-K-pump increases the consumption of ATP so much that at high levels of stimulation, it is necessary to get more glucose into the cell to manufacture more ATP.

Since insulin decreases Nai, elevates pHi, and increases production of ATP, one would expedt that the decreased insulin, such as seen in diabetes or in fasting would result in the reverse of these changes. Lowering plasma levels of insulin in rats by small doses of streptozotocin or by fasting produced an increase in Nai of about 30%, a decrease in pHi of 0.15 units, and a 24% decrease in ATPi. The action of thiazide diuretics to cause type 2 diuretics has been shown to be due to their ability to cause potassium loss. Not surprisingly, diets with large amounts of potassium and small amounts of sodium have been shown to reverse diabetes.

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Platelet Activating Factor Stimulates Sodium-Proton Exchange

Yoichi Ajiro[1,2], Noriko Saegusa[2], Kazunori Iwade[1], Wayne R. Giles[3], Diana Stafforini[4], Kenneth W. Spitzer[2].
[1]National Hospital Organization, Yokohama Medical Center, Kanagawa prefecture, Japan, [2]Nora Eccles Harrison Cardiovascular Research and Training Institute and Department of Physiology, University of Utah, Salt Lake City, UT, USA, [3]Department of Kinesiology, University of Calgary, Calgary, AB, Canada, [4]NHuntsman Cancer Institute, University of Utah, Salt Lake City, UT, USA.

Sodium-hydrogen exchanger (NHE), the principal sarcolemmal acid extruder in ventricular myocytes is stimulated by a variety of autocrine/paracrine factors and contributes to myocardial injury and arrhythmias during ischemia/reperfusion (IR). Platelet-activating factor (PAF, 1-O-alkyl-2-acetyl-sn-glycero-3-phosphocholine) is a potent proinflammatory phospholipid that is released in the heart in response to oxidative stress and promotes myocardial IR injury. PAF stimulates NHE in neutrophils and platelets, but its effect on cardiac NHE (NHE1) is resolved. We utilized quiescent guinea pig ventricular myocytes bathed in icarbonate-free solutions and used epifluorescence to measure intracellular pH (pHi). Methylcarbamyl-PAF (C-PAF, 200 nM), a metabolically-stable analog of PAF, significantly increased steady-state pHi. The alkalosis was completely blocked by the NHE inhibitor, cariporide, and by sodium-free bathing solutions, indicating it was mediated by NHE activation. C-PAF also significantly increased the rate of acid xtrusion induced by intracellular acidosis. The ability of C-PAF to increase steady-state pHi was completely blocked by the PAF receptor inhibitor WEB 2086 (10 μM), indicating the PAF receptor is required. A mitogen-activated protein (MAP) kinase kinase (MEK) inhibitor (PD98059, 25μM), also completely blocked the rise in pHi induced by C-PAF, suggesting participation of the MAP kinase signaling cascade downstream of the PAF receptor. Inhibition of protein kinase C (PKC) with GF109203X (1 μM) and chelerythrine (2 μM) did not significantly affect the alkalosis induced by C-PAF. In summary, these results provide evidence that: a) PAF stimulates cardiac NHE1, b) the effect occurs via the PAF receptor, and c) signal relay requires participation of the MAP kinase cascade.